42. (Reiterated) The method of claim 19, wherein each clone contains DNA obtained from a single organism.

43. (Reiterated) The method of claim 19, wherein the library is a multispecies library.

44. (Reiterated) The method of claim 43, wherein the library is generated from a mixed population of uncultured organisms.

45. (Reiterated) The method of claim 43, wherein the library is generated from isolates.

## REMARKS

### I. The Rejection under 35 U.S.C. § 112, Second Paragraph

Claim 19 stands rejected under 35 U.S.C. §112, second paragraph, as allegedly lacking antecedent basis for the phrase "the activity of interest". Applicants respectfully traverse this rejection.

Applicants have amended claim 19. Applicants respectfully submit that amended claim 19 has correct antecedent basis. Accordingly, Applicants respectfully request withdrawal of the rejection.

Claim 21 stands rejected under 35 U.S.C. §112, second paragraph, as allegedly lacking antecedent basis for the term "the enzyme". Applicants respectfully traverse this rejection.

Applicants have amended claim 21. Applicants respectfully submit that amended claim 21 has correct antecedent basis. Accordingly, Applicants respectfully request withdrawal of the rejection.

Claims 36 and 38 stand rejected under 35 U.S.C. §112, second paragraph, as allegedly lacking antecedent basis for the term "the enzyme". Applicants respectfully traverse this rejection.

Applicants have amended claims 36 and 38. Applicants respectfully submit that the amended claims properly recite correct antecedent basis. Accordingly, Applicants respectfully request withdrawal of the rejection.

Claim 36 stands rejected under 35 U.S.C. §112, second paragraph, as allegedly lacking antecedent basis for the phrase "the mutagenized DNA". Applicants respectfully traverse this rejection.

Claim 36 has been amended. Applicants respectfully submit that claim 36 properly recites correct antecedent basis. Accordingly, Applicants respectfully request withdrawal of the rejection.

Claims 38 and 41 stand rejected under 35 U.S.C. §112, second paragraph, as allegedly lacking antecedent basis for the phrase "the non-mutagenized DNA". Applicants respectfully traverse this rejection.

Applicants have amended claims 38 and 41. Claim 38 and 41 satisfy antecedent basis of the recited terms. Accordingly, Applicants respectfully request withdrawal of the rejection.

Claim 40 stands rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite because the term "at least one mutation" does not properly set forth the metes and bounds of the present invention. Applicants respectfully traverse this rejection.

Claim 40 has been amended to more clearly set forth Applicants' invention. The claims now recites "at least one nucleotide mutation". Thus, one of skill in the art will recognize that the term encompasses one nucleotide substitution, deletion or insertion as well as a mutation containing one or more nucleotide substitutions, deletions of insertions. Accordingly, Applicants respectfully request withdrawal of the rejection.

Claims 32 and 39 stand rejected under 35 U.S.C. §112, second paragraph as allegedly unclear due to the recitation of "stably inserted." Applicants respectfully traverse this rejection.

Claims 32 and 39 have been amended and do not recite "stably inserted". Accordingly, Applicants respectfully request withdrawal of the rejection.

## II. REJECTIONS UNDER 35 U.S.C. §112, FIRST PARAGRAPH

Claim 21 stands rejected under 35 U.S.C. §112, first paragraph because the specification, while being enabling for a method of screening DNA to identify lipases, esterases, glycosidases, proteases, and monooxygenases allegedly does not provide enablement for the use of such screening methods to identify glycosyl transferases, phosphatases, kinases, diarylpropane peroxidases, epoxide hydrolases, nitrile hydratases, nitrilases, transmainases, amidases and acylases.

The Office Action alleges that fluorescent substrates for all the enzymes listed in claim 21 are not enabled by the present specification. The Examiner has invited Applicants to identify the fluorescent substrates for glycosyl transferases, phosphatases, kinases, diarylpropane peroxidases, eposide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases, and acylases.

Applicants submit that there are numerous publications demonstrating enzymes and fluorescent substrates as well as methods of making fluorescent substrates known in the art. Such publications demonstrate that the skill in the art at the time the present application was filed. In addition, Applicants submit that those of skill in the art can practice the invention using the guidelines provided by the Specification without undue experimentation. Applicants disclose use of substrates that are fluorescent in the presence of the enzymatic activity of glycosyl transferases, phosphatases, kinases, diarylpropane peroxidases, epoxide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases and acylases (Specification, pages 34-39). The technique for using such substrates in practice of the invention methods does not differ substantially

from that illustrated in the Examples wherein the enzymes of interest are different, e.g., lipases, esterases, glycosidases, etc).

In addition, Applicants disagree with the Examiner's assertion that the relative skill of those in the art is low regarding selection of substrates that can be catalyzed by a particular enzyme as an indicator of enzymatic activity. Applicants respectfully submit that, in fact, substrates that fluoresce in the presence of the enzymatic activity of glycosyl transferases, phosphatases, kinases, diarylpropane peroxidases, epoxide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases and acylases were well known in the art at the filing date of the present application as identified by the attached references. Applicants respectfull remind the Examiner tha the specification need not and prefereably omits

Therefore, Applicants request reconsideration and withdrawal of the rejection of claim 2 for alleged lack of enablement under 35 USC §112, First Paragraph.

## III. The Rejection under 35 U.S.C. § 102(e)

The rejection of claims 1-18 under 35 U.S.C. § 102(e) for alleged anticipation by Thompson et al., (U. S. Patent No. 5,824,485, hereinafter "'Thompson") is respectfully traversed. Applicants' invention method for identifying bioactivity or biomolecule using high throughput screening, as defined by claim 19, distinguishes over the disclosure of Thompson by requiring screening of a library containing a plurality of clones obtained from more than one organism (e.g., a mixed population of uncultured or isolated organisms). Generally, each clone of the library contains DNA from a single organism within the mixed population. Thus, Applicants' method involves cloning of individual genes or groups of genes (e.g., pathways) obtained from an organism, into a host cell. The clones in Applicants' library include host cells containing DNA (e.g., single genes or pathways) obtained from an organism that may encode one gene product or more than one gene product.

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration (In re Spada, 15 USPQ 2d 1655 (Fed. Cir. 1990), In re Bond, 15 USPQ 2d 1566 (Fed. Cir., 1990).

By contrast to Applicants' invention method, the libraries described by Thompson are "combinatorial natural pathway expression library" containing "expression constructs prepared from genetic material obtained from one or more species of donor organisms in which genes present in the genetic material are operably associated with regulatory regions that drive[s] expression of the genes in an appropriate host organism" (Thompson, Col. 9, lines 27-32). In many cases, the clones do not each contain DNA from a single organism, but are synthetically produced, for example, by replacing one or more genes of a known pathway from organism X with one or more genes from the same pathway from organism Y in order to artificially create "new" pathways. Alternatively, one or more genes from pathway X may be replaced with one or more genes from pathway Y. In other examples provided by Thompson, a single host cell, which might contain a known gene A, from organism A, can be transformed with gene B from organism B and gene C from organism C, thereby producing a novel metabolic pathway. These novel pathways are synthetically produced and contain genes combined from various organisms. Thus, Thompson teaches a "combinatorial library" containing genomic DNA in which individual genes from different species can be concatenated in such a way as to produce a novel, non-naturally occurring pathway. However, Thompson is silent regarding preparation of a library of naturally occuring genes or gene pathways in which each clone may contain any type of DNA and wherein the DNA in each clone is obtained from an organism from a mixed population of organisms. Therefore, Thompson fails to teach each and every element of Applicants' method for high throughput screening as would be required to show anticipation under 35 USC§ 102(e).

Moreover, Applicants submit that Thompson also fails to suggest a method for high throughput screening of "natural" libraries under 35 USC § 103 to identify a bioactivity or a biomolecule of interest. In particular, Thompson fails to suggest that a library containing a plurality of clones obtained from more than one organism, but wherein each clone contains DNA

from one organism could be used to discover such bioactivities as result from natural but previously unknown pathways.

In high throughput screening of complex environmental libraries to identify genes encoding bioactivities or biomolecules of interest, the rate limiting steps in discovery occur at both the DNA cloning level and at the screening level. For example, a complex environmental library may contain hundreds of different organisms, requiring the analysis of several million clones. Such a library can be screened with relative ease by using Applicants' method because each clone contains the DNA derived from one organism in the multispecies population.

In addition, Applicants' method enhances the discovery of bioactivities within a complex environmental library containing, for example, DNA from thousands of different organisms. Applicants disclose a method for screening anywhere from about 30 million to about 200 million clones per hour for a desired biological activity so that naturally occurring pathways expressing novel biomolecules can be identified without any requirement for a preselection or combinatorial rearrangement of genes of a known pathway producing a known biomolecule. Rather, the method of the invention allows identification of, in many cases, previously unknown, naturally occurring pathways and genes and the biomolecules produced therefrom.

The method of the invention requires a substrate which is able to enter the cell and maintain its presence within the cell for a period sufficient for analysis to occur. It has generally been observed that introduction of the substrate into the cell across the cell membrane occurs without difficulty. It is preferable that once the substrate is in the cell it not "leak" back out before reacting with the biomolecule being sought to an extent sufficient to produce a detectable response. Retention of the substrate in the cell can be enhanced by a variety of techniques. In one disclosed by Applicants, the substrate compound is structurally modified by addition of a hydrophobic tail (e.g., see claim 31). Alternatively, preferred solvents, such as DMSO or glycerol, can be administered to coat the exterior of the cell. Also the substrate can be

administered to the cells at reduced temperature, which has been observed to retard leakage of the substrate from the cell's interior. A broad spectrum of substrates can be used in practice of the invention method which are chosen based on the type of bioactivity sought. The substrate is selected to interact with the target biomolecule to produce a detectable response. Applicants submit that Thompson fails to suggest methods useful for causing a fluorescent molecule to enter cells in a library and remain within the cells for a period of time sufficient to conduct high throughput screening of large libraries of molecules.

For the reasons discussed above, Applicants submit that Thompson neither anticipates nor renders obvious the invention as defined by new claims 19-42. Accordingly, Applicants respectfully request that this rejection be withdrawn.

## IV. The Rejection under 35 U.S.C. § 103(a)

**A.** Applicants respectfully traverse the rejection of claims 1 and 2 under 35 U.S.C. § 103(a) for allegedly being obvious over Thompson as applied in Section III above in view of Nadar et al. (U.S. Patent No. 5,173,187; hereinafter "Nadar"). Applicants respectfully disagree with the Examiner's assertion:

> It would have been obvious to one of ordinary skill in the art at the time the invention was made to screen an expression library, as taught by Thompson et al., for esterase activity using a fluorescent substrate, as taught by Nadar et al. One of ordinary skill in the art is motivated to do this for the benefit of identifying a clone of the expression library which expresses an esterase.

(Office Action, page 6.

As discussed above in Section III, Applicants' invention method for identifying an activity of interest using high throughput screening of DNA, as required by present claim 19 (and claims 20-42 dependent thereon), distinguishes over the disclosure of Thompson by screening of a library containing a plurality of clones derived from more than one organism.

The disclosure of Nadar fails to overcome these deficiencies in Thompson. Like Thompson, Nadar is completely silent regarding screening of a library containing a plurality of clones derived from more than one organism wherein each clone contains DNA derived from a single organism. Instead, Nadar discloses identification of species of bacteria in sludge. Accordingly, Applicants respectfully submit that the combined disclosures of Thompson and Nadar are not sufficient to establish the prima facie obviousness of Applicant's invention as defined by present claims 20 and 21 (which correspond to original claims 1 and 2).

**B.** Applicants respectfully traverse the rejection of claims 1 and 3 under 35 U.S.C. § 103(a) for allegedly being obvious over Thompson. As discussed above in Section III, Applicant's invention method for identifying a bioactivity or biomolecule of interest using high throughput screening of DNA, as required by present claim 19 (and claims 20-42 dependent thereon), clearly distinguishes over the disclosure of Thompson. Applicant submits that new claim 23, which contains all the limitations of claim 19 from which it depends, further distinguishes over Thompson in that it contains the further requirement of original claim 3 that the prokaryotic library contains "at least about 2 x $10^6$ clones." Therefore, Applicant submits that Thompson fails to teach or suggest Applicant's invention of new claim 23.

**C.** Applicants respectfully traverse the rejection of claims 1, 3, 10, 11 and 12 under 35 U.S.C. § 103(a) for allegedly being obvious over Thompson in view of Miao et al. (*Biotechnology and Bioengineering* 42:708-715, 1993; hereinafter "Miao"). Applicant respectfully disagrees with the Examiner's assertion:

> It would be a reasonable expectation to be able to use FACS screening of an expression library, as shown by the teachings of Thompson et al., with the fluorescent β-galactosidase substrate C12FDG, as shown by the teachings of Miao et al., to isolate β-galactosidase expressing clones as the teachings of Miao et al. show using fluorescence flow cytometry analysis of *E. coli* using C12FDG. Additionally, C12FDG comprises a lipophilic tail.

(Office Action, pages 10-11).

The deficiencies of Thompson for disclosing or suggesting Applicants' invention method for identifying an bioactivity or biomolecule of interest using high throughput screening of DNA, as required by present claim 19 (and claims 20-42 dependent thereon) are discussed in Section III above . Applicant respectfully submits that Miao fails to cure the deficiencies of Thompson. Miao's disclosure pertains to use of C12FDG as a fluorescent substrate in FACS screening of single bacterial cells of one species (i.e., E. coli). The focus of Miao's study is optimization of substrate concentration. However, like Thompson, Miao is completely silent regarding screening of a library containing a plurality of clones obtained from one or more organism wherein each clone contains DNA of a single organism. Accordingly, Applicant respectfully submits that the combined teachings of Thompson and Miao as alleged by the Examiner, including Miao's disclosure regarding C12FDG, are not sufficient to teach or suggest Applicant's invention of new dependent claims 22, 31, 32 and 33, which contain the requirements, respectively, of original claims 3, 10, 11 and 12.

**D.** Applicant respectfully traverses the rejection of claims 1 and 17 under 35 U.S.C. § 103(a) for allegedly being obvious over Thompson. As discussed above in Section III, Applicant's invention method for identifying a bioactivity or biomolecule of interest using high throughput screening of DNA, as required by present claim 19 (and claims 20-42 dependent thereon), clearly distinguishes over the disclosure of Thompson. Applicant submits, therefore, that any disclosure of Thompson pertaining to activities that the Examiner alleges are analogous to biopanning is not sufficient to render obvious Applicant's invention of new claim 39, which contains the additional requirement of original claim 17 that the expression library is biopanned prior to stable insertion of the substrate. Therefore, Applicant respectfully submits that Thompson fails to teach or suggest Applicant's invention of new claim 39.

**E.** Applicant respectfully traverses the rejection of claims 1 and 13-15 under 35 U.S.C. § 103(a) for allegedly being obvious over Thompson as applied above in view of Miao. With regard to Miao, the Examiner states:

> Miao et al. teach ("Abstract," page 708; "Single-Cell β-galactosidase activity by fluorescence flow cytometry using the fluorescent substrate C12FDG and growing E. coli cells at 37°C for a few minutes to an hour to allow permeation and reaction of the fluorescent substrate. Miao do not teach a method of screening an expression library or heating cells at 70°C. ...It would have been obvious to one of ordinary skill in the art to heat the samples at a temperature required for activity of the enzymes being screened.

(Office Action, page 12).

As discussed above in Paragraph B of this section, Applicant's invention method for identifying a bioactivity or biomolecule of interest using high throughput screening of DNA, as required by present claim 19 (and claims 20-42 dependent thereon), clearly distinguishes over the disclosure of Thompson. The disclosure of Miao does not overcome these deficiencies of the primary reference with regard to the identifying a bioactivity or biomolecule of interest using high throughput screening of DNA, as required by present claim 19. Miao is completely silent regarding screening of a library containing a plurality of clones obtained from one or more organism wherein each clone contains DNA from one organism in the multispecies population. Accordingly, Applicant respectfully submits that the combined teachings of Thompson and Miao, including Miao's disclosure regarding heating of the cells ,as alleged by the Examiner are not sufficient to disclose or suggest Applicant's invention as defined by new claims 33 and 34, which correspond to original claims 13 and 15.

Therefore, Applicants submit that new claims 19-42 are not obvious under 35 U.S.C.§ 103 over the disclosure of Thompson or the disclosure of Thompson in combination with that of Nadar or Miao.

In view of the above amendments and remarks, reconsideration and favorable action on ~~now~~ claims 19-45 are respectfully requested. In the event any matters remain to be resolved in view of this communication, the Examiner is encouraged to call the undersigned so that a prompt disposition of this application can be achieved.

Respectfully submitted,

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**EXHIBIT A**
**CLAIMS UPON ENTRY OF THE AMENDMENT**

19. (Amended) A method for identifying a bioactivity or biomolecule of interest using high throughput screening of DNA comprising:

a) contacting a bioactive substrate that is fluorescent in the presence of the bioactivity or biomolecule of interest with a library containing a plurality of clones containing DNA from more than one organism;

b) screening the library with a fluorescent analyzer that detects bioactive fluorescence, and

c) identifying clones detected as positive for bioactive fluorescence, wherein fluorescence is indicative of DNA that encodes a bioactivity or biomolecule of interest.

20. (Reiterated) The method of claim 19, further comprising obtaining DNA from a clone that is positive for an enzymatic activity of interest.

21. (Amended) The method of claim 20, wherein the enzymatic activity of interest is from an enzyme selected from the group consisting of lipases, esterases, proteases, glycosidases, glycosyl transferases, phosphatases, kinases, diarylpropane peroxidases, epoxide hydrolases, nitrile hydratases, nitrilases, transaminases, amidases, and acylases.

22. (Reiterated) The method of claim 19, wherein the library is generated in a prokaryotic cell.

23. (Reiterated) The method of claim 22, wherein the library contains at least about $2 \times 10^6$ clones.

24. (Reiterated) The method of claim 22, wherein the prokaryotic cell is gram negative.

25. (Reiterated) The method of claim 19, wherein the clones are encapsulated in a gel microdrop.

26. (Reiterated) The method of claim 19, wherein the analyzer screens up to about 15 million clones per hour.

27. (Reiterated) The method of claim 19, wherein the clones are extremophiles.

28. (Reiterated) The method of claim 27, wherein the extremophiles are thermophiles.

29. (Reiterated) The method of claim 27, wherein the extremophiles are hyperthermophiles, psychrophiles, halophiles, psychrotrops, alkalophiles, or acidophiles.

30. (Reiterated) The method of claim 19, wherein the bioactive substrate comprises staining reagent C12FDG.

31. (Reiterated) The method of claim 19, wherein the bioactive substrate comprises a lipophilic tail.

32. (Amended) The method of claim 19, wherein the clones and substrates are heated to enhance contacting of the substrate with the clones.

33. (Reiterated) The method of claim 32, wherein the heating is to a temperature of about 70°C.

34. (Reiterated) The method of claim 32, wherein the heating is for about 30 minutes.

35. (Reiterated) The method of claim 19, wherein the fluorescent analyzer comprises a fluorescence activated cell sorting (FACS) apparatus.

36. (Amended) The method of claim 20, wherein the enzymatic activity of interest encoded by the DNA is stable at a temperature of at least about 60°C.

37. (Reiterated) The method of claim 19, wherein the library is an expression library.

38. (Amended) The method of claim 20, wherein the enzymatic activity of interest encoded by the DNA possesses enhanced enzymatic activity of interest compared to that of a wild-type enzyme.

39. (Amended) The method of claim 19, wherein the method further comprises biopanning the expression library prior to contacting with the substrate.

40. (Amended) The method of claim 19 further comprising obtaining DNA from a clone identified in step c) that is positive for an enzymatic activity of interest and comparing the enzymatic activity of a DNA expression product from the clone with that obtained from such a clone into whose DNA at least one nucleotide mutation has been introduced, wherein a difference in enzymatic activity is indicative of the effect upon the enzymatic activity of interest caused by introduction of the at least one nucleotide mutation.

41. (Amended) The method of claim 19, wherein the bioactivity encoded by the DNA possesses the bioactivity of interest at a temperature at least 10°C below the temperature of optimal activity of the bioactivity encoded by the wild-type DNA.

42. (Reiterated) The method of claim 19, wherein each clone contains DNA obtained from a single organism.

43. (Reiterated) The method of claim 19, wherein the library is a multispecies library.

44. (Reiterated) The method of claim 43, wherein the library is generated from a mixed population of uncultured organisms.

45. (Reiterated) The method of claim 43, wherein the library is generated from isolates.